WHAT IS CLAIMED IS:

1   **1.**   A method of detecting a target species immobilized on a substrate,

2   said method comprising:

3   detecting a single copy of said target species by detecting fluorescence

4   emitted by a quantum dot attached to said single copy, wherein said single copy is bound

5   to an affinity moiety for said target species immobilized on said substrate.

1   **2.**   The method according to claim **1**, wherein said quantum dot is

2   attached to said target species prior to binding said target species to said affinity moiety.

1   **3.**   The method according to claim **1**, wherein said quantum dot is

2   attached to said target species after binding said target species to said affinity moiety.

1   **4.**   The method according to claim **1**, wherein said target species has a

2   second quantum dot attached thereto and said first quantum dot is distinguishable from

3   said second quantum dot.

1   **5.**   The method according to claim **4**, wherein binding of said target

2   species to said affinity moiety forms a target species-affinity moiety complex that is

3   detected by fluorescence from both said first quantum dot and said second quantum dot

4   attached to said target species-affinity moiety complex.

1   **6.**   The method according to claim **4**, wherein said first quantum dot

2   and said second quantum dot are distinguishable by a characteristic which is a member

3   selected from the group consisting of fluorescence spectrum, fluorescence emission,

4   fluorescence excitation spectrum, ultraviolet light absorbance, visible light absorbance,

5   fluorescence quantum yield, fluorescence lifetime, light scattering and combinations

6   thereof.

1   **7.**   The method according to claim **4**, wherein said first quantum dot

2   and said second quantum dot are visually distinguishable as a first color and a second

3   color, respectively.

1   **8.**   The method according to claim **7**, wherein said first color and said

2   second color combine to form a visually or electronically distinguishable color different

3   from both said first color and said second color.

1   **9.**   The method according to claim **1**, wherein said target species has n
2 quantum dots attached thereto, wherein each of said n quantum dots is distinguishable
3 from each other, and n is an integer from 3 to 10.

1   **10.**   The method according to claim **1**, wherein said first quantum dot is
2 attached to a targeting moiety for said target species, said targeting moiety being a
3 member selected from the group consisting of antibodies, , aptamers, proteins,
4 streptavidin, nucleic acids and biotin.

1   **11.**   The method according to claim **1**, wherein said affinity moiety is
2 labeled with a quantum dot.

1   **12.**   The method according to claim **1**, wherein said target species is a
2 member selected from the group consisting of organisms, biomolecules and bioactive
3 molecules.

1   **13.**   The method according to claim **1**, wherein said affinity moiety is a
2 member selected from the group consisting of organisms, biomolecules and bioactive
3 molecules.

1   **14.**   The method according to claim **1**, wherein said substrate has bound
2 thereto a second affinity moiety.

1   **15.**   The method according to claim **14**, wherein said first affinity
2 moiety and said second affinity moiety are different affinity moieties.

1   **16.**   The method according to claim **1**, wherein said substrate has bound
2 thereto m affinity moieties; and m is an integer from 1 to 10,000.

1   **17.**   The method according to claim **16**, wherein each of said m affinity
2 moieties is a different affinity moiety.

1   **18.**   The method according to claim **16**, wherein said m affinity
2 moieties are ordered in an array format.

1   **19.**   The method according to claim **1**, wherein said substrate further
2 comprises an alignment moiety providing a reference point on said substrate for the

3   detection of a target-affinity moiety complex formed between said target and said affinity

4   moiety, wherein said target-affinity moiety complex is distributed upon said substrate in a

5   random manner, said alignment moiety comprising a fluorescent label, which does not

6   interact with said target species or said affinity moiety.

1   **20.**   The method according to claim **19**, wherein said alignment moiety

2   comprises a quantum dot.

1   **21.**   The method according to claim **19**, wherein said alignment moiety

2   is distinguishable from each quantum dot attached to said target species.

1   **22.**   The method according to claim **19**, wherein said alignment moiety

2   is correlated with the position of one or more target moiety-affinity complexes.

1   **23.**   The method according to claim **1**, wherein said substrate is

2   manufactured from a low fluorescence optical material configured as a member selected

3   from the group consisting of a microtiter plate, a glass slide, a microscope slide cover

4   slip, a capillary, a flow cell, a bead and combinations thereof.

1   **24.**   The method according to claim **1**, further comprising, counting

2   each detected quantum dot per unit area of said substrate, producing substrate quantum

3   dot data; and comparing said substrate quantum dot data with standard quantum dot

4   quantity data acquired from a standard of said quantum dot having a known

5   concentration, thereby quantifying said target species immobilized on said substrate.

1   **25.**   A data set comprising data acquired by a method according to

2   claim **1**.

1   **26.**   The data set according to claim **25**, wherein said data set is in an

2   electronic format.

1   **27.**   A computer disc having information stored thereon, said

2   information comprising said data set according to claim **26**.

1   **28.**   A database comprising two or more data sets according to claim

2   **25**, wherein said database is in a searchable format.

29. A method of detecting a target species in solution, said method comprising:

detecting a single copy of said target species by detecting essentially simultaneously fluorescence emitted by a first quantum dot of a first color attached to said single copy and a second quantum dot of a second color attached to said single copy, wherein said first color and said second color are distinguishably different colors.

30. A method of detecting a target species immobilized on a substrate, which species is a member of a population of target species immobilized on said substrate with spacing between each member of said population, said method comprising:

detecting a single copy of said target species by detecting fluorescence emitted by a quantum dot attached to said single copy, wherein said single copy is bound to an affinity moiety for said target species immobilized on said substrate, wherein said detecting is performed with a detecting means having a resolution that is higher than said spacing between each member of said population.

1 **31.** A method of detecting a target species immobilized on a substrate,
2 which species is a member of a population of target species immobilized on said
3 substrate, said method comprising:
4 detecting a single copy of said target species by detecting fluorescence
5 emitted by a quantum dot attached to said single copy, wherein said single copy is bound
6 to an affinity moiety for said target species immobilized on said substrate forming a
7 target-affinity moiety complex, and said detecting is performed with a detecting means
8 having a resolution limited region of interest such that, in general, less than one target-
9 affinity moiety complex is present within each resolution limited region of interest.

1 **32.** A method of detecting a first target species immobilized on a
2 substrate, which species is a member of a population of target species immobilized on
3 said substrate, said method comprising:
4 (a) defining a first region of interest of said substrate;
5 (b) probing said first region of interest for fluorescence emitted by a
6 quantum dot attached to a single copy of said first target species
7 bound to an affinity moiety for said first target species immobilized
8 on said substrate, wherein said probing resolves said fluorescence

9   from said first target species from fluorescence arising from other

10   members of said population of target species immobilized on said

11   substrate.

1   **33.**   The method according to claim **32**, further comprising detecting a

2   second target species immobilized to said substrate, said method comprising:

3   (c) defining a second region of interest of said substrate; and

4   (d) probing said second region of interest for fluorescence emitted by a

5   quantum dot attached to said a single copy of said second target

6   species bound to an affinity moiety for said second target species

7   immobilized on said substrate, wherein said probing resolves said

8   fluorescence from said second target species from fluorescence

9   arising from other members of said population of target species

10   immobilized on said substrate.

1   **34.**   The method according to claim **33**, wherein said first region of

2   interest and said second region of interest are the same region of interest.

1   **35.**   The method according to claim **32**, wherein said probing is by a

2   method selected from the group consisting of microscopy, confocal fluorescence

3   microscopy and two-dimensional imaging with a CCD camera.

1   **36.**   The method according to claim **32**, wherein said first target species

2   and said second target species are different species.

1   **37.**   A method for detecting multiple target species immobilized on a

2   substrate, which species are members of a population of target species immobilized on

3   said substrate, said method comprising:

4   (a) defining multiple regions of interest on said substrate; and

5   (b) probing said multiple regions of interest for fluorescence emitted by a

6   quantum dot attached to a single copy of said target species bound

7   to an affinity moiety for said target species immobilized within a

8   region of interest of said substrate, wherein said probing resolves

9   fluorescence from said multiple target species from other members

10   of said population and from each other.

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1   **38.**   A method for determining whether a target species within a region

2   of interest on a substrate is quantifiable by a technique selected from the group consisting

3   of single target counting and ensemble counting, said method comprising:

4   (a) probing said region of interest to determine target species density

5   within said region of interest by detecting fluorescence emitted by

6   a quantum dot attached to one or more molecules of said target

7   species bound to an affinity moiety for said target species

8   immobilized on said substrate;

9   (b) comparing said density to a predetermined density cutoff value above

10   which ensemble counting is used and below which single target

11   counting is used.

1   **39.**   The method according to claim **38**, wherein said substrate

2   comprises a first region in which ensemble counting is used and a second region in which

3   single target counting is used.